Reference: *Biol. Bull.* **181**: 127–134. (August, 1991)

Alloimmunity in the Gorgonian Coral *Swiftia exserta*

LUISA SALTER-CID AND CHARLES H. BIGGER

Department of Biological Sciences, Florida International University, Miami, Florida 33199

Abstract. This study of histocompatibility demonstrates that the gorgonian *Swiftia exserta* (Coelenterata, Anthozoan) fulfills the minimal functional criteria of cytotoxicity, specificity, and altered secondary response (memory) that characterize an adaptive immune response. All autografts (self grafts) fused, and all allografts (intraspecific grafts) underwent rejection, which is characterized by rapid and progressive blanching, necrosis, and loss of tissue in the immediate contact area. Initial reactions required 7–9 days to produce 1 mm of necrosis, but after a resting period, a second contact at a new tissue area yielded the same reaction in 3–4 days. After primary sensitization, intervals of up to eight weeks still produced a significantly accelerated secondary response. Significant differences between the reaction times of second set and third party allografts demonstrated recognition specificity in these responses. Thus, this is the first report of an adaptive alloimmune response in gorgonians.

Introduction

Most immunologists would agree that specificity and memory are the hallmarks of adaptive immune reactivity. According to some authors (Hildemann *et al.*, 1979), only the following functional criteria are necessary to define such immunological competence: (1) antagonistic or cytotoxic reaction after sensitization; (2) selective or specific reactivity; and (3) inducible memory, *i.e.*, selectively altered (positive or negative) reactivity on secondary contact.

Possession of a specific adaptive immune system, including immunorecognition leading to selectively inducible responses with a memory component, was, until recently, considered to be restricted to vertebrates (Klein, 1989). Invertebrate defense mechanisms, often associated with phagocytosis and encapsulation, were thought to lack sharp specificity. We now know that at least some metazoan invertebrates, ranging from sponges to protochordates, possess a well-developed capacity for allogeneic recognition followed by incompatibility reactions (see Bigger, 1988).

Some definitive studies have been carried out on invertebrates. Relatively short-term memories were found in the sponge *Callyspongia diffusa* (4 weeks; Bigger *et al.*, 1982) and the coral *Montipora verrucosa* (8 weeks; Hildemann *et al.*, 1980a), but a longer term memory was reported for allograft rejection in the sea urchin, *Lytechinus pictus* (6 months; Coffaro, 1980). Specific immune memory, however, has not been found in all invertebrates even within the same class or phylum. For example, immune memory has been reported to be absent in some sponges (*e.g.*, Van de Vyver, 1980; Smith and Hildemann, 1984) and earthworms (Parry, 1978). Therefore, without testing, we cannot assume that the details of an immune response in one species are transferable to other members of the phylum.

The gorgonians (Anthozoa; Alcyonaria) are an Order of soft corals that includes sea fans and sea whips. Theodor's extensive studies (Theodor, 1966, 1969, 1970, 1976; Serre and Theodor, 1967; Theodor and Carriere, 1975; Theodor and Senelar, 1975) of *Eunicella stricta* and *Lophogorgia sarmentosa*, and the preliminary work of Bigger and Runyan (1979) on *Leptogorgia virgulata*, *Pseudopterogorgia elisabethae* and *Plexura flexulosa*, have identified some of the major characteristics of histocompatibility in the Order. Naturally occurring grafts within the same colony (autografts) occur in field, and all experimentally induced autografts rapidly fuse within 24 h. (Theodor, 1970; Bigger and Runyan, 1979). Rapid xenogeneic reactions (between two individuals of different species) were the focus of Theodor's study of gorgonian histocompatibility; he hypothesized a system of "induced suicide" underlying graft rejection. The delay in the cytotoxic responses of

Received 2 May 1990; accepted 1 April 1991.

Abbreviations: RT—reaction time, MRT—mean reaction time, 1°—first (primary) graft, 2°—secondary graft, 3P—third party graft.

allografts (between two individuals of the same species) suggested, to Theodor, fundamental differences in the mechanisms operative in xenogeneic *versus* allogeneic cytotoxic incompatibilities. Gorgonian histoincompatibility mechanisms have not been characterized at the cellular and molecular levels. As for immunocompetence, the gorgonians certainly fulfilled the first criterion of cytotoxic alloincompatibility; but prior to this study, the parameters of rejection and other aspects, such as those of memory and specificity, had not been tested or were not found.

The purpose of this project was to study tissue incompatibility reactions between different colonies of the gorgonian coral *Swiftia exserta* (Fig. 1). We asked whether the corals possess attributes of an adaptive immune system.

Materials and Methods

Animal collection and maintenance

Swiftia exserta individuals were purchased from various sources and were collected in the waters off Southeast Florida, at depths of approximately 30 m. The animals were transported to Florida International University, where they were maintained in 25 or 100 gallon seawater aquaria with sub-gravel filters, and fed newly hatched *Artemia*.

All aquaria were subjected to alternating periods of 12 hours of light and dark. The water temperature was generally maintained at 20–22°C with aquarium heaters, although during the summer, the temperatures occasionally rose to 25°C. Individual experiments were confined to a range of about 1°C or less. Salinity values ranged from 31 to 34 0/00. Because all treatments in an experiment were run concurrently in the same aquarium, all were subjected to the same general conditions. Although there were several colonies in each aquarium, they were not in contact. The animals were acclimated for at least 4 days before being placed in experimental contact. As opposed to many other gorgonians, *Swiftia exserta* lives well under laboratory conditions. Also, as we report in this study, its rapid allogenic reactions make it especially attractive for immunological studies.

Techniques of grafting and scoring

Small branches, 2–3 cm, were clipped from a gorgonian colony with surgical scissors and immediately placed in either allogeneic (intraspecific) or isogeneic (= autograft) combinations. To eliminate any influence of size on the reactions, all pairings involved tissue pieces of about the same size. The two pieces were gently placed in close contact without traumatizing the cell surfaces. We call this grafting. Unless otherwise stated, secondary grafts (2°) were performed after a sensitization period of three days; *i.e.*, the tissues were put in contact for three days and then separated for some given length of time before regrafting. A three-day period of sensitization was chosen to assure full maturation of the immune response; *i.e.*, at this time cytotoxic reactions were underway in all graft pairs but not completed.

A system of third-party grafting (3P) was used to test for specificity in the allograft response. It was performed like the secondary graft except that tissue from an allogeneic animal not used in the sensitization was separately grafted with each of the test tissues for the second interaction. A lack of specificity should result in all allogeneic tissue being treated the same, and that would be reflected in similar second set and third-party rejection times. Specificity would be demonstrated if the third party grafts were treated in a naive fashion.

The coral branches were immobilized in small holders, each consisting of a 4 mm diameter × 5–6 cm length of glass tube bent into a "v" shape. The ends of the glass tube were covered with Nalgene 8000 plastic tubing (1/8″ ID × 1/16″ wall) 1–1.5 cm long. Small cuts (2 mm) were made in the plastic into which the coral branches were inserted (Fig. 2). A 2–3 mm long section of tissue was removed from the axial skeleton at the cut end of the branch that was inserted into the tubing; this operation precluded the necrosis that is induced when the tissue is pinched. Contact was between intact surfaces rather than the cut-ends of the branch.

Tip to side, side to side, and tip to tip cytotoxic reactions were similar in direction and appearance, and paired *t*-tests between reaction times showed no significant differences between the rates (data not shown). Therefore, the reaction is independent of the site chosen for pairing. In tip to tip, and some tip to side grafts, the support was unstable, and the individual pieces separated before the reaction endpoint was reached. No response would occur in such cases, because rejection seems to depend on unbroken physical contact. Because no significant influence of tissue locality was observed, side to side grafting was used in all the subsequent experiments and technical problems were minimized.

After grafting, the individuals were examined daily under a dissecting microscope at 40× magnification. One of two responses occurred: either *fusion*—complete growth together of the tissues of the two branches (Fig. 3), or *rejection*—tissue necrosis of one or both individuals at the contact zone between the two colonies (Fig. 4).

Cytoxic reactions were arbitrarily scored as definitive when soft tissue destruction extended 1.0 mm or more to either side of an interface. The time required, in days, to reach that point was called the reaction time (RT). The reaction is easy to score for this species because the white necrotic tissue stands out in marked contrast to the orange

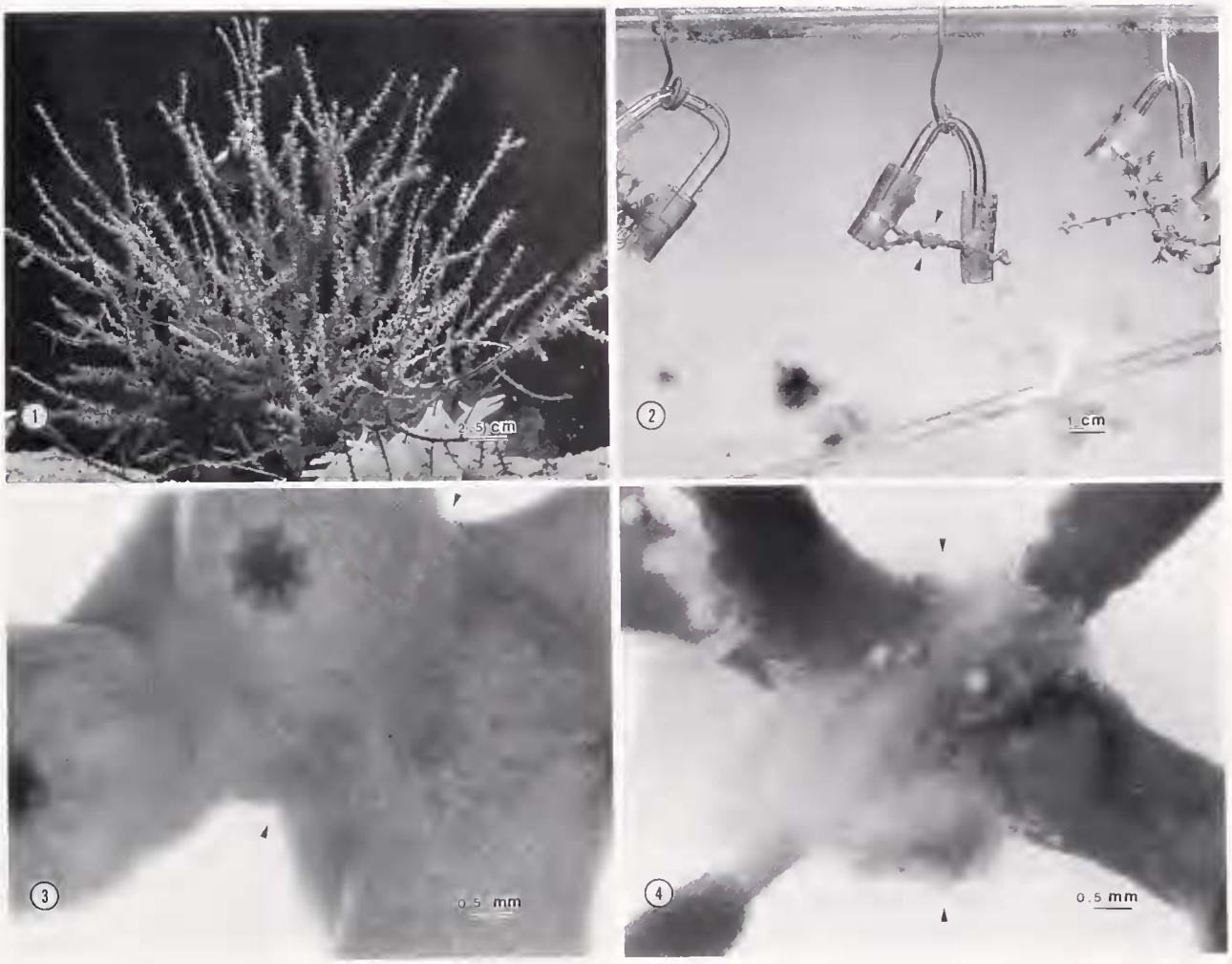

Figure 1. Colony of *Swiftia exserta*.

Figure 2. Technique of pair grafting. Coral pieces cut from the same or separate colonies of *S. exserta* are immobilized by the holders with their contact surfaces intimately opposed (between arrows).

Figure 3. Intracolony autografts of *S. exserta* showing compatible fusion at interface (arrows).

Figure 4. Intracolony allografts of *S. exserta* showing cytotoxic incompatibility restricted to the immediate contact zone. Note blanching and soft tissue death at interface (arrows) and exposed axial skeleton.

color of the living soft tissues. Because as little as 0.1 mm of necrosis can usually be discriminated, end points can be determined quantitatively.

Data analysis

Mean reaction times (MRT) and their standard deviations in days were determined. Paired *t*-tests were used to determine the significance of the differences between the experimental and control data because the animals, in both cases, were genetically identical.

Results

Pathology

Grafts between parts of the same colony were always compatible. Soft tissues fused in the area of contact as early as one day after grafting. Neither tissue bleaching nor necrosis accompanied fusion in any of the ten pairs tested (Fig. 3). Compatible fusion persisted indefinitely. The fused branches could not be pulled apart easily; the tissues always tore before separating.

Rejection of initial allografts was preceded by a lag of 3–5 days; during this time intimate tissue contact was required. Progressive allograft rejection occurred in the following sequence: (1) tissue blanching caused by the disappearance of pigmentation in the immediate contact zone; (2) loss of spicules from the tissues; and (3) tissue death eventually leading to exposure of the hard axial skeleton (Fig. 4). Intensified reactivity (more rapid and acute necrosis) was accompanied by secretion of mucus at the interface. On two occasions, overgrowth of one col-

Table I

Timing of initial allosensitization or immune induction in Swiftia exserta

Duration of initial allogeneic contact (a) (days)	No. of coral pairs tested (n)	Graft (b)	Mean reaction time (days) (c)	Range of indiv. react. times (days)	Difference between 1st and 2nd reaction times (d)
1	45	ctl.	8.3 ± 0.5	7–9	N.S.—$P \geq 0.4$
		test	8.2 ± 0.7	7–9	
2	45	ctl.	8.0 ± 0.4	7–9	SIG—$P \leq 0.001$
		test	3.3 ± 0.5	3–4	
3	45	ctl.	8.3 ± 0.6	7–9	SIG—$P \leq 0.001$
		test	3.7 ± 0.5	3–4	

(a) Sensitization by first-set allografting was allowed for 1, 2, or 3 days at 22–24°C; coral pairs were then each regrafted at a new interface 3 days later.

(b) Concurrent controls (primary grafts) were performed with the same combinations as the test grafts.

(c) Mean ± standard deviation of the mean.

(d) N.S.—not significant. SIG—significant at the 0.1% level. Significance was determined by the paired *t*-test.

ony by the other at one or both sides of the interface was observed; this overgrowth was concurrent with the usual necrosis.

Parameters of graft response

Reproducibility of reaction times. The first experimental set consisted of grafting 10 replicates of each of 10 allogeneic combinations and 8 replicates of each of 10 isogeneic combinations. The very similar reaction times (RTs), and standard deviations that were less than one day for each combination, justified our use of a single graft of each combination in all subsequent treatments (data not shown).

Alloimmune memory. In a series of 45 test pairs, control primary allografts yielded a mean reaction time of 8.3 ± 0.6 days. Secondary allograft tests, established by resetting the same coral pairs at new interfaces away from the original contact area following a three-day separation, showed accelerated and intensified cytotoxic reactions with a MRT of 3.7 ± 0.5 days (Table I). The MRT difference, with an interval of three days between first-set and second-set grafting, was highly significant ($P < 0.001$), indicative of immune memory. Intensified second-set reactivity was characterized by an earlier onset and stronger necrosis and secretion of mucus at the interfaces.

Three days of presensitization were successful in eliciting an accelerated second-set reaction. Possible earlier induction of memory was tested by disjoining initial allografts after one or two days of contact and regrafting the same pairs at new interfaces three days later (Table I).

Coral pairs regrafted after only 1 day of allogeneic contact yielded a MRT of 8.2 days with a range of individual cytotoxic reaction times of 7–9 days (Table I). Thus, no significant difference was observed between the RTs of the control and test pairs. As shown in Table I, after 2 days of presensitization, all second-set grafts already displayed accelerated activity with a MRT of 3.3 ± 0.5 days. Paired *t*-tests showed that the difference between the primary and secondary RTs was significant ($P < 0.001$).

Two days of tissue contact were sufficient to elicit accelerated allograft responses. Longer sensitization periods did not improve the response time, and the sensitization appeared to be "all or nothing" rather than a gradual transition.

To test for specificity, 90 pairs of third-party grafts were performed. The protocol is shown in Figure 5. Rather than responding in a purely naive or sensitized manner, these third-party grafts yielded a broad range of cytotoxic reaction times reflecting a distribution of accelerated to non-accelerated responses as is shown by the range of individual reaction times of 4–9 days (Table II). The reaction time of third-party grafts (mean 5.9 days) was significantly different from both first and second-set graft reaction times (8.2 days and 3.5 days respectively, Table II).

To test for long-term memory, groups of grafts were reset at new interfaces at 2, 4, 6, and 8 weeks after separation from primary contact. The resulting MRTs, standard deviations, and the range of individual cytotoxic reaction times, are given in Table III. Strong alloimmune memory was present in the second-set group grafted 2 weeks after first-set separation, as shown by an early MRT of 4.1 days ± 0.5 days and a significant difference ($P < 0.001$) between the primary and secondary RTs. This sensitized state was still present after 4 weeks, with an MRT of 3.6 ± 0.6 days. At 6 weeks after first-set sepa-

ration, the accelerated reaction started to fade, as is shown by the MRT of 6.4 and the individual reaction times of 5–8 days, but is still significant ($P < 0.001$). This fading of alloimmune memory was still more accentuated at the 8-weeks period, with a MRT of 6.9 ± 0.9 and an individual range of 6–9 days. Although there is some degree of overlapping in the values of the control and test grafts from the 8-week group, the difference between the primary and secondary RTs is still significant ($P < 0.001$); the response gets progressively weaker in all combinations approaching the timing of a naive response.

Discussion

This study demonstrates that the anthozoan gorgonian *Swiftia exserta* displays: (a) cytotoxic reactivity accompanying allogeneic incompatibility, (b) early and vigorous primary allograft rejection, and (c) selective alloimmunity with a specific memory component. Thus, *S. exserta* fulfills the three criteria for an adaptive immunological response, described by Hildemann *et al.* (1979).

Among other coelenterates, allogeneic incompatibilities have been documented for hydrozoans (*e.g.*, Ivker, 1972; Buss *et al.*, 1984) and anthozoans, including gorgonians (*e.g.*, Theodor, 1970), sea anemones (*e.g.*, Bigger, 1980), and scleractinian corals (*e.g.*, Hildemann *et al.*, 1977). Except for the coral *Montipora verrucosa* (Hildemann *et al.*, 1980a), however, no substantial data bearing on the existence or persistence of immune memory in this phylum has heretofore been available.

In the gorgonian coral, *Swiftia exserta*, autografts fuse compatibly, but allografts were invariably incompatible. The pattern of necrosis (Fig. 4) suggests that the response is triggered by a cellular process rather than a diffusible factor; *i.e.*, because tissue destruction was limited to the graft interface, either cell contact or a very short-range cytotoxic molecule was responsible for the cytotoxic response. *Swiftia exserta* is an attractive model for tissue

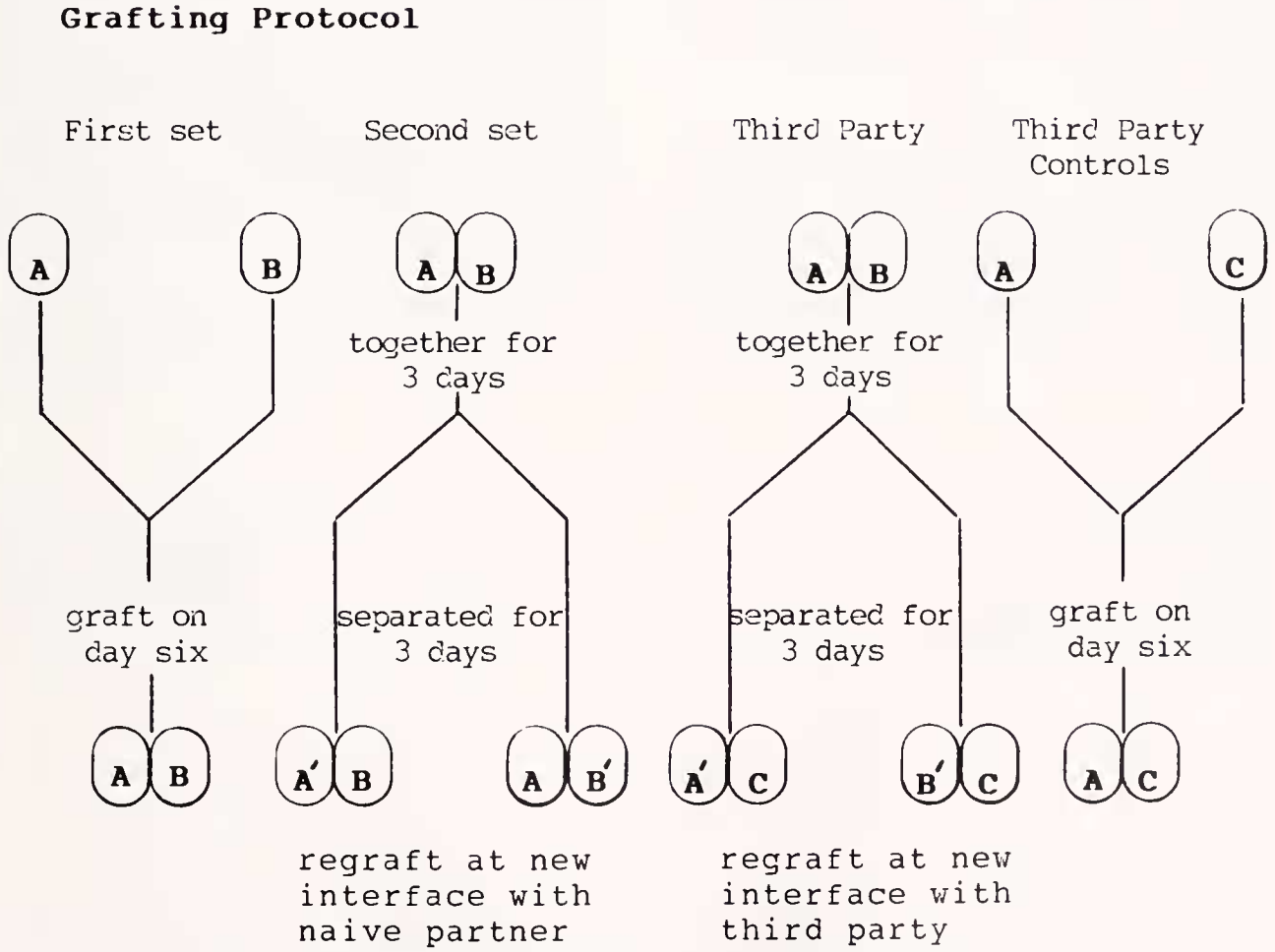

Figure 5. Experimental protocols used in the specificity study (Table II). A, B, and C stand for naive tissue from colonies (clones). A, B, and C. A′ and B′ denote presensitized individuals from the same respective colonies. 1°—first-set; 2°—second-set; 3P—third party; ctl—third-party controls.

Table II

Specificity of allograft reaction times in Swiftia exserta

Grafts (a)	No. of coral pairs tested (n)	Mean reaction time (b) (days)	Range of individual reaction times (days)
First set	45	8.2 ± 0.6	7–9 (c)
Second set	90	3.5 ± 0.4	3–4 (c)
Third party	90	5.9 ± 1.5	4–9 (c)
Control	90	8.3 ± 0.6	7–9 (c)

(a) Concurrent controls were performed with the same combinations as the third party grafts.

(b) Mean ± standard deviations of the mean.

(c) The difference between second-set and third-party (3P) graft responses and the difference between 3P and control graft responses are both highly significant ($P \leq 0.001$, paired t test).

Experimental temperature range: 21–24°C.

grafting, because the responses are easily scored and the rapid rates of rejection (primary MRTs of about 7 to 10 days) are closer to those observed in mammalian transplantation than in the coral *M. verrucosa* (MRTs of about 18 to 22 days, Hildemann *et al.*, 1980a).

As Burnet (1969) pointed out, recognition of self/not-self is the cornerstone of immunological recognition. It has been suggested that while vertebrates recognize not-self, invertebrate responses are based on recognition of self (*e.g.*, Burnet, 1971). Although *S. exserta* shows a range of reaction times and severity, the results from a given combination of colonies were highly reproducible. This pattern of differing responses between different allogeneic combinations and the specificity associated with memory, *i.e.*, that different allogeneic "not-self" elicits different responses, supports the opposite hypothesis, that allogeneic rejection is based on a recognition of "not-self" (see Bigger, 1988; Neigel, 1988). Among 1479 alloparabiotic combinations of the gorgonian *Eunicella stricta* collected in the Banyuls-sur-mer (France) area, Theodor (1976) observed only 11 or 0.7% compatible fusions, whereas the remaining 99.3% exhibited rejection reactions. Even the small number of compatible gorgonians could have originated from the same clone, because all the specimens were collected from the same area. That impressive variation of histocompatibility markers, as well as that found between the 10 colonies employed in the present study, suggest that each separate clone or colony has a unique array of alloimmunorecognition molecules, as predicted by the concept of the "uniqueness of the individual" developed by Medawar (*e.g.*, Jokiel *et al.*, 1983).

The early acquisition of immune memory in other corals (Hildemann *et al.*, 1980a) seemed to be a gradual or quantitative process, with accelerated reactivity present after 2 to 4 days of contact and maximal presensitization developed after 4 to 8 days; that activation or positive memory then persisted or diminished only slightly after prolonged primary contact. In *S. exserta*, because there is apparently no significant difference between the MRTs of the secondary responses elicited after 2 or 3 days of primary contact (3.3 and 3.7 d respectively, Table I), it seems to be an all or none process occurring at approximately 2 days.

The present study demonstrates that significant short-term alloimmune memory persists for at least 8 weeks,

Table III

Duration of Swiftia exserta *alloimmune memory*

Time interval between 1st sensitization and 2nd graft (weeks)	No. of coral pairs tested (n)	Graft (a)	Mean reaction time (b) (days)	Range of indiv. react. times (days)	Significance of the difference between the primary and secondary reaction times (c)
2	43	ctl.	8.1 ± 1.0	7–10	SIG
		test	4.1 ± 0.5	3–4	
4	45	ctl.	7.9 ± 0.8	7–9	SIG
		test	3.6 ± 0.6	3–5	
6	42	ctl.	7.8 ± 0.8	7–9	SIG
		test	6.4 ± 1.0	5–8	
8	39	ctl.	7.9 ± 0.8	7–9	SIG
		test	6.9 ± 0.9	6–9	

(a) Concurrent controls (primary grafts) were performed with the same combination as the test grafts.

(b) Mean ± standard deviation of the mean.

(c) Significance was determined by the paired t-test and in all cases represents $P \leq 0.001$.

but starts to fade at 6 weeks in the gorgonian coral *S. exserta* (Table III). As with similar short-term immune memory in the sponge *C. diffusa* (Bigger *et al.*, 1982), annelids (Dales, 1978), and echinoderms (Coffaro, 1980), this appears to constitute a major difference from the long-term alloimmune memory found in mammals (Hildemann, 1984). In mice and rats, immune memory demonstratable by accelerated rejection of test skin allografts resides in lymphocytes and persists more than one year after sensitization (Billingham *et al.*, 1963). It is important to note however, that in this study, no tests were performed after prolonged sensitization (> 3 days) and that there was no investigation of environmental factors such as temperature, light, and salinity, which may affect the effectiveness of sensitization and the cytotoxic reaction times (*e.g.*, Johnston *et al.*, 1981). Further investigation of conditions that might favor long-term memory is desirable, because the absence of this characteristic could be a major distinguishing feature of invertebrates. Memory that lasts only weeks (Bigger *et al.*, 1982), rather than months or years (Billingham *et al.*, 1963), could have several causes. This immunologic feature in sponges and corals may be the result of shorter-lived memory residing in immunocytes with commensurate life spans (Hildemann *et al.*, 1980b). For example, the sensitized immunocytes may be replaced by naive cells. Alternatively, the molecules in which memory is imprinted may become inactive with time or with immunocyte division. Killer cells or molecules involved with memory have yet to be identified as a special subset in any coelenterate (see Bigger and Hildemann, 1982). Thus, these studies of memory must await further progress.

Only 27% of the third-party individuals reacted as fast as the slowest of the second-set allografts, while the remainder responded in a similar fashion to primary grafts or at intermediate times, demonstrating a specificity involved in the secondary response. These results contrast with the ones obtained by Hildemann *et al.* (1980a) in *M. verrucosa*. A bimodal distribution of third-party reaction times such that approximately 66% of the third-party individuals reacted as fast as the second-sets led them to suggest the occurrence of cross reactivity. The present study presents a different situation; third-party reaction times were not bimodal and, while there is a demonstrable specificity, an allogeneicly non-specific effect appears to be manifested in the third-party allografts. Alternatively, another explanation is that the accelerated responses of some third-party individuals could be the result of the sharing of a limited repertoire at minor histocompatibility loci (locus), which might exist together with a not-shared, highly polymorphic MHC. However, there is no genetic data that pertains. Why third-party grafts reacted in a significantly different way from primary as well as secondary grafts must await further investigation.

This study supports the hypothesis (*e.g.*, Burnet, 1976) that invertebrates may be capable of anticipatory immune responses, as opposed to suggestions (Klein, 1989) that, whereas vertebrate immune responses are based on recognition of not-self, invertebrate responses are not anticipatory and are non-specific. Unfortunately, the study of immunity in invertebrates has been restricted so far to very few animals, and most of these have been investigated in little detail; therefore, many questions remain unanswered. *Swiftia exserta* provides a very good model in which to study these invertebrate defense mechanisms, which need clarification.

Acknowledgments

Thanks are due to Ms. Cecile Olano for providing photographic assistance, Ms. Lois Bigger for editorial assistance, and anonymous reviewers for their helpful comments. Support was provided by NIH grant RR08205.

Literature Cited

Bigger, C. H. 1980. Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones, a recognition of self and not-self. *Biol. Bull.* **159:** 117–134.

Bigger, C. H. 1988. Historecognition and immunocompetence in selected marine invertebrates. Pp. 55–65 in *Invertebrate Historecognition*, R. Grosberg, D. Hedgecock, and K. Nelson, eds. Plenum Press, New York.

Bigger, C. H., and W. H. Hildemann. 1982. Cellular defense systems of the coelenterata. Pp. 59–87 in *The Reticuloendothelial System*, N. Cohen and M. M. Sigel, eds. Plenum Publishing Corp., New York.

Bigger, C. H., P. L. Jokiel, W. H. Hildemann, and I. S. Johnston. 1982. Characterization of alloimmune memory in a sponge. *J. Immunol.* **129:** 1570–1572.

Bigger, C. H., and R. Runyan. 1979. An *in situ* demonstration of self-recognition in gorgonians. *Dev. Comp. Immunol.* **3:** 591–594.

Billingham, R. E., W. K. Silvers, and D. B. Wilson. 1963. Further studies on adaptive transfer of sensitivity to skin homografts. *J. Exp. Med.* **118:** 397–401.

Burnet, F. M. 1969. *Self and Not-Self: Cellular Immunology, Book 1.* Melbourne and Cambridge University Presses, Carlton, Victoria, and London.

Burnet, F. M. 1971. "Self-recognition" in colonial marine forms and flowering plants in relation to the evolution of immunity. *Nature* **232:** 230–235.

Burnet, F. M. 1976. The evolution of receptors and recognition in the immune system. Pp. 35–58 in *Receptors and Recognition I*, P. Cuatrecasa and M. F. Greaves, eds. Chapman and Hall, London.

Buss, L. W., C. S. McFadden, and D. R. Keene. 1984. Biology of hydractinid hydroids. 2. Histocompatibility effector system/competitive mechanism mediated by nematocyst discharge. *Biol. Bull.* **157:** 139–158.

Coffaro, K. A. 1980. Memory and specificity in the sea urchin *Lytechinus pictus*. Pp. 77–88 in *Phylogeny of Immunological Memory*, M. J. Manning, ed. Elsevier/North Holland, New York.

Dales, R. P. 1978. The basis of graft rejection in the earthworms *Lumbricus terrestris* and *Eisenia foetida*. *J. Invertebr. Pathol.* **32:** 264–268.

Hildemann, W. H. 1984. A question of memory. *Dev. Comp. Immunol.* **8:** 747–756.

Hildemann, W. H., C. H. Bigger, and I. S. Johnston. 1979. Histoincompatibility reactions and allogeneic polymorphism among invertebrates. *Transplant Proc.* **11:** 1136–1141.

Hildemann, W. H., P. L. Jokiel, C. H. Bigger, and I. S. Johnston. 1980a. Allogeneic polymorphism and alloimmune memory in the coral *Montipora verrucosa*. *Transplantation* **30:** 277–301.

Hildemann, W. H., C. H. Bigger, P. L. Jokiel, and I. S. Johnston. 1980b. Characteristics of immune memory in invertebrates. Pp. 9–14 in *Phylogeny of Immunological Memory*, M. J. Manning, ed. Elsevier/North Holland, New York.

Hildemann, W. H., R. L. Raison, G. Cheung, C. J. Hull, C. Akabe, and J. Okanoto. 1977. Immunological specificity and memory in a scleractinian coral. *Nature* **270:** 219–223.

Ivker, F. B. 1972. A hierarchy of histoincompatibility in *Hydractinia echinata*. *Biol. Bull.* **143:** 162–174.

Johnston, I. S., P. L. Jokiel, C. H. Bigger, and W. H. Hildemann. 1981. The influence of temperature on the kinetics of allograft reactions in a tropical sponge and a reef coral. *Biol. Bull.* **160:** 280–291.

Jokiel, P. L., W. H. Hildemann, and C. H. Bigger. 1983. Clonal population structure of two sympatric species of the reef coral *Montipora*. *Bull. Mar. Sci.* **33:** 181–187.

Klein, J. 1989. Are invertebrates capable of anticipatory immune responses? *Scand. J. Immunol.* **29:** 499–505.

Neigel, J. E. 1988. Recognition of self or non-self? Theoretical implications and an imperical test. Pp. 127–142 in *Invertebrate Historecognition*, R. Grosberg, D. Hedgecock, and K. Nelson, eds. Plenum Press, New York.

Parry, M. J. 1978. Survival of body wall autografts, allografts and xenografts in the earthworm *Eisenia foetida*. *J. Invertebr. Pathol.* **31:** 383–392.

Serre, A., and J. Theodor. 1967. Mise en évidence d'une reconnaissence immunologique de tisus chez un invertébré. *C.R. Acad. Sc. Paris.* **264:** 513–514.

Smith, L. C., and W. H. Hildemann. 1984. Alloimmune memory is absent in *Hymeniacidon sinapium*, a marine sponge. *J. Immunol.* **133:** 2351–2355.

Theodor, J. 1966. Contribution à l'étude des Gorgones (V) les greffs chez les gorgones: étude d'un système de reconnaissence du tissus. *Bull. Inst. Oceanogr. Monaco.* **66:** 1–7.

Theodor, J. 1969. Histotoxicité *in vivo* et *in vitro* entre tissus xénogéniqus et entre tissus allogénipus chez un invertébré. *C. R. Acad. Sc. Paris.* **268:** 2534–2535.

Theodor, J. 1970. Distinction between "self" and "not-self" in lower invertebrates. *Nature.* **227:** 690–692.

Theodor, J. 1976. Histoincompatibility in a natural population of gorgonians. *Zool. J. Linnean Soc.* **58:** 173–176.

Theodor, J., and J. Carriere. 1975. Direct evidence of heterolysis of gorgonian target cells. Pp. 101–104 in *Immunologic Phylogeny*, W. H. Hildemann and A. A. Benedict, eds. Plenum Press, New York.

Theodor, J., and R. Senelar. 1975. Cytotoxic interaction between gorgonian explants: mode of action. *Cell. Immunol.* **19:** 194–200.

Van de Vyver, G. 1980. Second-set allograft rejection in two sponge species and the problem of an alloimmune memory. Pp. 15–25 in *Phylogeny of Immunological Memory*, M. J. Manning, ed. Elsevier/North Holland, New York.